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- (71) Applicant (*for all designated States except US*): **MAR-LOW FOODS LIMITED** [GB/GB]; Station Road, Stokesley, Cleveland TS9 7AB (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **FINNIGAN, Timothy, John, Andrew** [GB/GB]; 1 Rudby Lea, Hutton Rudby, North Yorkshire TS15 0JZ (GB). **BLANCHARD, Robin** [GB/GB]; 23 West End, Stokesley, North Yorkshire TS9 5BL (GB).
- (74) Agents: **NEILL, Alastair, William et al.**; Appleyard Lees, 15 Clare Road, Halifax HX1 2HY (GB).
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(54) Title: EDIBLE FUNGI

(57) Abstract: An aqueous formulation comprising edible fungal particles especially consisting substantially of fungal mycelia is described. The ingredient may be combined with other ingredients to produce a wide range of foodstuffs or food ingredients including desserts (e.g. yoghurt), reconstitutable drinks or soup and extruded foodstuffs (e.g. savoury snack foods). Foodstuffs prepared may have medical applications (e.g. for treatment of joint mobility disorders, reducing fat uptake, lowering cholesterol, immune function stimulation, use on a pre-biotic and/or for affecting satiety).

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EDIBLE FUNGI

This invention relates to edible fungi and provides a method of preparing edible fungi for use in foodstuffs, formulations of edible fungi, dry particles comprising edible fungi, uses and methods associated with the aforesaid, foodstuffs per se and foodstuffs, methods and uses of edible fungi in the promotion of good health.

10 It is known, for example from WO 00/15045 (DSM), WO96/21362 (Zeneca) and WO95/23843 (Zeneca) to use edible filamentous fungi as meat-substitutes, for example in the preparation of burgers and sausages. In such uses, filaments of the fungi are bound together, for example
15 with egg albumin, and are texturised so that the product resembles muscle fibres and therefore has a meat-like appearance and texture. Meat substitutes of the type described have been widely commercially available for many years under the trade mark QUORN.

20

The present invention, in one aspect, is based on the discovery that edible fungi can be arranged to act as fat mimetics (in sharp contrast with known uses where they are arranged to be meat-like and mimic muscle fibres) and be
25 used in a range of foodstuffs with excellent consumer acceptability.

It is also well-known to deliver active ingredients (e.g. vitamins, minerals, pharmaceuticals etc) in tablet
30 (or other dosage) forms. Active ingredients may be prepared synthetically, then isolated and tableted. Alternatively, active ingredients may be extracted from raw materials containing them and then tableted. It is

also known to fortify foods with active ingredients (e.g. vitamins). However, in the aforesaid cases, a concentrate of substantially pure active ingredient is incorporated into the food, at low concentration and so as to have
5 negligible effect on the functionality, taste and/or rheology of the food. Disadvantageously, the preparation of concentrates of active ingredients can be expensive. Furthermore, it is difficult to deliver sufficiently high levels of a range of desired active ingredients without
10 detrimentally affecting the quality of the food.

The present invention, in another aspect, is based on the discovery of a means of delivering active ingredients into certain foodstuffs at levels at which they can
15 provide positive health benefits and/or promote good health. Furthermore, at the same time, the means of delivering the active ingredients can replace ingredients (e.g. fat) in foodstuffs that may potentially be detrimental to good health and contribute positively to
20 the functionality and/or rheology of the foodstuff.

Thus, it is an object of the present invention to provide foodstuffs which may be advantageous over known foodstuffs.

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According to a first aspect of the invention, there is provided a method of preparing an aqueous formulation of edible fungi, the method comprising providing a mixture which includes edible fungi in an aqueous liquid and
30 subjecting the mixture to a size reduction process in order to produce an aqueous formulation comprising edible fungal particles having a dimension in a first direction

of less than 200 μ m, wherein said dimension in said first direction is a maximum dimension of said particles.

Said edible fungi preferably comprise filamentous fungi. Said filamentous fungi preferably comprise fungal mycelia and suitably the edible fungi used in the method includes at least 80wt%, preferably at least 90wt%, more preferably at least 95wt% and, especially, at least 99wt% of fungal mycelia. Some filamentous fungi may include both fungal mycelia and fruiting bodies. Preferred filamentous fungi for use in the method do not produce fruiting bodies. Where, however, filamentous fungi of a type which produces fruiting bodies are used in the method, the edible fungi used in the method suitably includes at least 80wt%, preferably at least 90wt%, more preferably at least 95wt% of fungal mycelia. Preferably, substantially only the fungal mycelia are used in the method - that is, said edible fungi provided in said mixture preferably do not include any fruiting bodies.

20

Preferred fungi have a cell wall which includes chitin and/or chitosan. Preferred fungi have a cell wall which includes polymeric glucosamine. Preferred fungi have a cell wall which includes β 1-3/1-6-glucans.

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The edible fungi may include fungal cells of the order Mucorales as described in WO 00/15045 (DSM).

Said edible fungi is preferably selected from fungi imperfecti.

30

Preferably, the edible fungi comprise, and preferably consist essentially of, cells of *Fusarium* species,

especially of *Fusarium venenatum* A3/5 (formerly classified as *Fusarium graminearum*) (IMI 145425; ATCC PTA-2684 deposited with the American Type Culture Collection, 10801 University Boulevard Manassas, VA, US) as described for
5 example in WO96/21361 (Zeneca) and WO95/23843 (Zeneca).

Edible fungi provided in said mixture are preferably not bound together by a binding agent added to the fungi after they have been grown and/or harvested. Thus, said
10 edible fungi need not be treated with hydrocolloids (e.g. starch, pectin, carrageenan or alginate) and/or with proteins (e.g. milk protein such as casein, ovoprotein such as egg albumin or eggs themselves; vegetable proteins such as soy; cereal proteins, such as gluten; or enzymes
15 such as proteases or phosphodiesterases). It is especially preferred that said edible fungi are not bound together by egg albumin. Thus, said edible fungi need not be texturized prior to inclusion in said mixture.

20 Edible fungi in said mixture prior to said size reduction process preferably have a dimension in a first direction which is a maximum dimension of particles of said edible fungi, of at least 400 μm . The dimension in said first direction suitably refers to the length of
25 respective edible fungi (especially where the fungi are filamentous). Preferably, the number average dimension in said first direction, e.g. length, of said edible fungi (i.e. the sum of the dimensions in the first direction divided by the total number of fungi measured) in said
30 mixture before said size reduction process is at least 400 μm . The average dimension in said first direction (e.g. length) may be less than 1000 μm , preferably less than 800 μm .

Said mixture may include at least 2% w/w, suitably includes at least 3% w/w, preferably includes at least 5% w/w, more preferably includes at least 9% w/w of said edible fungi on a dry matter basis. Said mixture may
5 include less than 20% w/w, or less than 15% w/w of said edible fungi on a dry matter basis.

Said mixture may include at least 50% w/w, suitably at least 70% w/w, preferably at least 75% w/w, more
10 preferably at least 80% w/w water (including water present in any component of the mixture). In some cases, for example wherein the main or only solid material in the mixture is provided by edible fungi, said water content may be at least 85% w/w or even at least 89% w/w.

15

The water content is suitably less than 95% w/w, preferably less than 91% w/w. In cases wherein edible fungi are not the only solid material, the water content may be 88% w/w or less.

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Said aqueous liquid may comprise water having dissolved and/or suspended solids, for example as in milk, e.g. skim milk, or said aqueous liquid may consist essentially of water. In some embodiments said aqueous
25 liquid may include a protein, for example a vegetable protein such as pea protein isolate. In some embodiments, said aqueous liquid may include a range of ingredients (e.g. sugar, oil, thickener, stabiliser) which may be components of a final product which incorporates said
30 edible fungi. Where said aqueous liquid includes dissolved and/or suspended solids (in addition to said fungi) the amount of such solids in said mixture may be less than 10% w/w, preferably less than 7.5% w/w.

Said mixture may be prepared by contacting said edible fungi and said aqueous liquid. Said edible fungi are preferably in the form of a paste (that is comprising
5 solids and water). The paste may include at least 10% w/w, preferably at least 20% w/w edible fungi (e.g. fungal mycelia or hyphae) on a dry matter basis. The paste may include less than 50% w/w, preferably less than 40% w/w, more preferably less than 30% w/w of edible fungi on a dry
10 matter basis.

Preferably, after contact of said edible fungi and said aqueous liquid, the edible fungi are allowed to equilibrate with the aqueous liquid for at least 5
15 minutes, more preferably at least 30 minutes prior to said size reduction process.

Said size reduction process preferably involves the use of a size reduction apparatus which is able to subject
20 the mixture to high shear forces. Said size reduction process suitably does not include the use of a blade or blades arranged solely to affect the cutting of the edible fungi. In one embodiment, the size reduction apparatus may comprise a high shear blender. In another embodiment,
25 said apparatus may comprise a homogeniser. In general, the use of a homogeniser is preferred over the blender. In some embodiments, the size reduction process may use two size reduction apparatuses, suitably operated sequentially. For example, a said blender may be used,
30 followed by a said homogeniser. In some situations, for example when pilot plant (or larger) apparatus is used, shear forces generated within appropriately configured

process equipment may be sufficient to effect size reduction.

Preferably, said aqueous formulation prepared is
5 substantially homogenous.

As described above, the aqueous formulation produced in the process comprises particles having a dimension in a first direction of less than $200\mu\text{m}$. The maximum dimension
10 suitably refers to the length of the fungal particles (especially where the fungi are filamentous) but the reference to length is not intended to exclude the possibility of there being two (or more) substantially equal maximum dimensions which may extend perpendicularly
15 to each other. The number average of said first dimensions of solid fungal particles produced in the method is suitably less than $200\mu\text{m}$, is preferably less than $100\mu\text{m}$, is more preferably less than $75\mu\text{m}$ and is especially less than $50\mu\text{m}$. In some embodiments, said
20 number average may be less than $40\mu\text{m}$, less than $30\mu\text{m}$ or even less than $20\mu\text{m}$. The aforementioned smaller dimensions may be particularly useful for incorporation in certain foodstuffs.

25 The number average of said first dimensions may be at least $1\mu\text{m}$, preferably at least $5\mu\text{m}$, more preferably at least $10\mu\text{m}$.

Preferably, the ratio of the number average of said
30 first dimensions of the particles after said size reduction to the number average of said first dimensions of the fungi before said size reduction is less than 0.5, preferably less than 0.25, more preferably less than 0.1.

Suitably, the mean of said first dimensions is less than 150 μ m, preferably less than 100 μ m, more preferably less than 75 μ m with a standard deviation on the mean of less than 200 μ m, preferably less than 100 μ m. The mean is preferably at least 10 μ m.

Said edible fungal particles (after said size reduction) may have a dimension in a second direction, measured perpendicular to said first direction, which is suitably less than 20 μ m, preferably less than 10 μ m, more preferably less than 7 μ m and especially 5 μ m or less. Said dimension in said second direction is preferably at least 1 μ m, more preferably at least 3 μ m. Said dimension in said second direction is preferably a diameter of the particles and is preferably substantially the same as a dimension in a third direction, perpendicular to the dimension in said second direction. Thus, preferably said particles have a substantially circular cross-section.

Preferably, the number average of the dimensions of the fungal particles in said second direction is substantially the same for the edible fungi and the particles before and after the size reduction process.

The method of the first aspect may include contacting edible fungi with said aqueous liquid. The edible fungi may be in the form of a hydrated mass (e.g. a paste) which may include at least 50% w/w, suitably at least 60% w/w, preferably at least 70% w/w water. Said hydrated mass suitably includes at least 10% w/w, preferably at least 15% w/w, more preferably at least 20% w/w, especially at least 23% w/w of edible fungi on a dry matter basis. Said

amount of edible fungi may be less than 40% w/w, preferably less than 30% w/w on a dry matter basis.

Said aqueous liquid may include at least 80% w/w, preferably at least 90% w/w water. In some embodiments, the aqueous liquid consists essentially of water. In other embodiments, said aqueous liquid may be milk, suitably having less than 15% w/w, preferably less than 10% w/w of milk solids on a dry matter basis. The amount of milk solids may be at least 5% w/w, preferably at least 7.5% w/w on a dry matter basis.

According to a second aspect of the invention, there is provided a method of preparing an aqueous formulation of edible fungi, the method comprising providing a mixture which includes edible fungi in an aqueous liquid and subjecting the mixture to a size reduction process in order to produce an aqueous formulation comprising edible fungal particles having an average aspect ratio of less than 70.

For the avoidance of doubt, the average aspect ratio suitably refers to the average of the dimensions of the fungal particles in a first direction (e.g. the average length) divided by the average of the dimensions of the fungal particles in a second direction (e.g. diameter).

Edible fungi in said mixture prior to said size reduction preferably have an average aspect ratio of at least 100, more preferably at least 150, especially at least 200. The average aspect ratio may be less than 500, preferably less than 300.

The average aspect ratio of the particles after said size reduction process is suitably less than 65, preferably less than 60, more preferably less than 50, especially less than 40. In some embodiments, the average
5 may be less than 30, less than 20, less than 15, less than 10 or even less than 5.

The aspect ratios of the second aspect may be applied to the invention of the first aspect.

10

According to a third aspect of the present invention, there is provided an aqueous formulation of edible fungi prepared according to the first and/or second aspects.

15 According to a fourth aspect of the present invention, there is provided an aqueous formulation of edible fungi, the formulation comprising edible fungal particles having a dimension in a first direction of less than 200 μ m wherein said dimension in said first direction is a
20 maximum dimension of said particles and/or an aspect ratio of less than 70 in an aqueous liquid.

Said aqueous formulation is preferably substantially homogenous.

25

Said formulation may includes at least 3% w/w, suitably includes at least 5% w/w, more preferably includes at least 7% w/w and especially includes at least 9% w/w of said edible fungi on a dry matter basis. Said
30 mixture may include less than 20% w/w, or less than 15% w/w of said edible fungi on a dry matter basis.

Said mixture may include at least 50% w/w, suitably at least 70% w/w, preferably at least 75% w/w, more preferably at least 80% w/w water (including water present in any component of the mixture). In some cases, for example wherein the main or only solid material in the mixture is provided by edible fungi, said water content may be at least 85% w/w or even at least 89% w/w.

The water content is suitably less than 95% w/w, preferably less than 91% w/w. In cases wherein edible fungi are not the only solid material, the water content may be 88% w/w or less.

Said formulation optionally includes milk solids (e.g. provided by skim milk). The formulation may include 0 to 15% w/w, suitably 0-10% w/w especially 0 to 7.5% w/w milk solids on a dry matter basis.

Said formulation is suitably shear thinning pseudoplastic, suitably exhibiting apparent viscosities ranging between 3000 and 20 centipoise over the range 2 to 100 rpm on a Brookfield LV1 rotational viscometer.

The aqueous formulation of the fourth aspect may have any relevant characteristic described according to the first and second aspects.

The aqueous formulation described herein may have many potential uses, for example in the preparation of foodstuffs (e.g. yoghurts, deserts, drinks) and/or ingredients for foodstuffs as hereinafter described. Advantageously, the formulation can be used to prepare foodstuffs which have a lower fat content than in

corresponding conventional foodstuffs, since the edible fungi when present in the form described have been found to act as a fat mimetic.

5 One use of the aqueous formulation is in the preparation of dried edible fungal particles having low or substantially no residual moisture. Therefore, according to a fifth aspect of the present invention, there is provided a method of preparing dried particles of edible
10 fungi which suitably may be used as a fat mimetic in downstream applications, the method comprising removing water from an aqueous formulation of the third or fourth aspects and isolating dried particles of said edible fungus.

15

The dimensions and/or average dimensions and/or aspect ratios of the dried particles are preferably as described herein for the particles in said aqueous formulation.

20 Dried particles isolated in the method may have a residual moisture content of less than 10% w/w, suitably less than 7.5% w/w, preferably less than 5% w/w, more preferably less than 3% w/w. The residual moisture content may be greater than 0.5% w/w.

25

The bulk density of the dried particles may be in the range 200-8000 kgm⁻³.

30 Preferably, water is removed in the method by spray drying the aqueous formulation.

In some circumstances, an aqueous formulation which includes milk, for example skim milk (rather than water

alone) together with edible fungal particles may be more advantageously dried than a formulation which does not include milk and/or includes water alone. More particularly, dry particles prepared from a formulation
5 which includes milk may be re-dispersible in an aqueous liquid more readily in downstream processing.

In the method of preparing dry particles, said aqueous formulation used may include at least 5% w/w, preferably
10 at least 7% w/w of edible fungi on a dry matter basis. The amount of edible fungi may be less than 15% w/w, for example less than 13% w/w.

According to a sixth aspect of the invention, there is
15 provided dry particles comprising edible fungi prepared in a method according to the fifth aspect.

According to a seventh aspect of the invention, there is provided dried particles comprising edible fungi per
20 se.

The dimensions and/or average dimensions and/or aspect ratios of the dried particles are preferably as described herein for the particles in the aqueous formulation. In
25 preferred embodiments, the number average of dimensions of fungal particles in a first direction wherein said first dimension is a maximum dimension of the particles is less than 50 μ m. The average aspect ratio may be at least 200.

30 According to an eighth aspect of the invention, there is provided the use of an aqueous formulation comprising edible fungi or dried particles comprising edible fungi as described herein in the preparation of a foodstuff.

According to a ninth aspect of the invention, there is provided a method of preparing a foodstuff, the method comprising contacting an aqueous formulation comprising edible fungi or dried particles comprising edible fungi as described herein with other ingredients of said foodstuff.

The aqueous formulation of edible fungi or dried particles of edible fungi may be as described in any statement herein. The amount of said aqueous formulation or dried particles of said edible fungi may be selected such that in the prepared foodstuff, there is at least 2% w/w, preferably at least 3% w/w, more preferably at least 4% w/w, especially at least 4.5 %w/w of edible fungi on a dry matter basis (especially fungal mycelia or hyphae). The amount of said edible fungi on a dry matter basis may be less than 10% w/w, suitably less than 8% w/w, preferably less than 7% w/w, more preferably less than 6% w/w, especially less than 5% w/w.

20

The other ingredients and the amounts thereof in said foodstuff will generally depend on the nature of the foodstuff being prepared. However, ingredients common to a number of foodstuffs are suitably milk (e.g. skim milk) and/or milk (e.g. skim milk) powder. Thus, the method may involve contacting the edible fungi with milk or milk powder wherein the amount of milk powder may be at least 2% w/w, preferably at least 3% w/w. It is preferably less than 20% w/w, more preferably less than 15% w/w. The amount of skim milk may be less than 80% w/w, preferably less than 75% w/w. Another ingredient that may be common to a number of foodstuffs is sugar (i.e. sucrose) and the method may involve contacting the edible fungi with sugar

wherein the amount of sugar is at least 0.5% w/w, suitably is at least 1% w/w preferably is at least 2% w/w, more preferably is at least 3% w/w and, especially, is at least 3.5% w/w. The amount may be less than 15% w/w, preferably
5 less than 13% w/w.

Said foodstuff may include a protein source, especially a vegetable-derived protein source such as pea protein. Such a protein source may be additional to but suitably is
10 used instead of milk or skim milk. Advantageously, the method may involve adding edible fungi or dried particles thereof to a dispersion or preferably a solution, suitably an aqueous solution of said protein, thereby to contact the ingredients. Suitably, the aqueous formulation
15 prepared in the method includes at least 1% w/w, preferably at least 2% w/w, more preferably at least 2.5% w/w, of protein from said protein source; and suitably includes less than 10% w/w, preferably less than 8% w/w, more preferably less than 7% w/w, especially less than 6%
20 w/w of edible fungi on a dry matter basis. Suitably, at least 2% w/w, preferably at least 3% w/w, more preferably at least 4% w/w, especially at least 4.5% w/w of edible fungi on a dry matter basis is in said aqueous formulation.

25

In some embodiments, said foodstuff may include no milk (dairy product) or skim-milk (dairy product) and, more preferably, includes no ingredient derived from milk. In this event, said foodstuff may advantageously address the
30 problem of lactose intolerance.

It has been found that the edible fungi can act as a fat mimetic and, accordingly, the amount of fat and/or fat

containing ingredients added can be reduced. More particularly, it has been found that the edible fungi promote the creamy mouthfeel typically associated with fat.

5

The foodstuff prepared in the method may be a dessert (e.g. a chilled dessert), for example a mousse, crème caramel or chocolate dessert (or the like). More generally, the foodstuff may be a hot-fill, cold-fill, 10 demouldable, non-demouldable, aerated or non-aerated dessert.

The foodstuff prepared in the method may be a yoghurt. In one embodiment, preparation of a yoghurt may involve 15 contacting, suitably with mixing, edible fungi (suitably in said aqueous formulation or as dry particles) with sugar, milk (e.g. skim milk) and/or milk (e.g. skim milk) powder and water. In another embodiment, preparation of a yoghurt may involve contacting edible fungi (suitably in 20 said aqueous formulation or as dry particles) with a protein source, for example a vegetable-derived protein source such as pea protein. The mixture may be subjected to a size-reduction process; for example sheared. Thereafter, sweetening means, for example sugar may be 25 added. The total protein content in the yoghurt may be at least 2% w/w, preferably at least 3% w/w, more preferably at least 4% w/w, especially at least 5% w/w. The amount may be less than 10% w/w, preferably less than 8% w/w, more preferably less than 6% w/w. After contact and 30 mixing of the ingredients, a culture may be added and the mixture incubated. Thereafter, the mixture may be sheared, prior to the optional addition of flavouring. Advantageously, less than 1% w/w suitably less than 0.5%

w/w, preferably less than 0.2% w/w, more preferably less than 0.1% w/w, especially substantially no additional polysaccharide and/or gelatin stabilisers are added to the yoghurt in the method.

5

The foodstuff prepared in the method may be an ice-cream type dessert. Preparation of a said dessert may involve contacting, suitably with mixing (e.g. with a high shear mixer), said edible fungi with sugar, glucose syrup, milk (e.g. skim milk) powder and oil (e.g. palm oil) and optionally one or more stabiliser/emulsifier. After further treatment, the mixture may be whipped and frozen.

The foodstuff prepared in the method may be a milk drink. In one embodiment, preparation of such a drink preferably involves the use of said edible fungi in combination with milk (e.g. skim milk) paste or powder. For example, the combination may comprise a dispersion of edible fungi in skim milk or a dispersion of dry particles comprising skim milk and fungi. The combination is preferably contacted with other ingredients and milk and/or water added as required with suitable mixing. In another embodiment, a milk drink may be prepared which is not dairy product based and suitably therefore does not include any dairy products. In this case, said foodstuff may be prepared by contacting edible fungi, suitably dry particles thereof, with an oil (e.g. a vegetable oil) and with water. A sweetener for example sucrose may also be added. A suitable stabiliser and/or thickener may be included. The mixture is preferably mixed to produce a substantially homogenous dispersion. Said foodstuff may include at least 1% w/w, preferably at least 2% w/w; and suitably 10% w/w or less, preferably 5% w/w or less of

edible fungi on a dry matter basis. Said foodstuff may include at least 0.5% w/w, preferably at least 1% w/w; and suitably less than 5% w/w, preferably less than 2.5% w/w of an oil. The foodstuff may include at least 85% w/w of
5 water.

The foodstuff prepared in the method may be a low fat spread which suitably comprises a water in oil emulsion wherein, suitably, the oil phase is a continuous phase and
10 the water phase is a dispersed phase. Preferably, the foodstuff is prepared such that the edible fungi are a component of the water phase. To this end, preferably the method involves contacting edible fungi, preferably an aqueous dispersion thereof, with other ingredients to
15 prepare the water phase. Preferably, a buttermilk solution is prepared which includes said edible fungi. Said preparation may include a homogenisation step. Other ingredients may be added into the water phase. An oil phase may be prepared in a conventional manner.

20

After preparation of the respective oil phase and water phase, the two are mixed and processed to prepare the spread.

25 According to a tenth aspect of the invention, there is provided a foodstuff which comprises edible fungi.

Said edible fungi in said foodstuff may have any feature of the edible fungi prepared in the first and/or
30 second aspects; and/or present in the aqueous formulations of the third and/or fourth aspects and/or resulting from a method according to the ninth aspect.

The ratio of the %w/w of egg albumin powder to the % w/w of edible fungi in said foodstuff is suitably less than 0.1, preferably less than 0.05, more preferably less than 0.01. Preferably the foodstuff includes
5 substantially no albumin powder and/or no egg albumin at all.

Said edible fungi are preferably adapted to act as a fat mimetic in the foodstuff.

10

Said foodstuff preferably includes a quantity of edible fungi on a dry matter basis as present in the foodstuff prepared in the ninth aspect. Said foodstuff may include 2-10%w/w, preferably 4-10% w/w of edible fungi
15 on a dry matter basis.

Said foodstuff preferably includes edible fungal particles having a dimension in a first direction of less than 200 μ m wherein said dimension in said first direction
20 is a maximum dimension of said particles and/or an aspect ratio of less than 70. The dimensions and/or aspect ratio may be as described in any statement herein.

Said foodstuff may be a dairy product.

25

Said foodstuff may be selected from the group comprising a dessert (e.g. yoghurt or ice-cream type dessert), milk drink (including non-dairy based drinks) or low-fat spread. Preferably, it is selected from a dessert
30 (e.g. yoghurt or ice-cream type dessert) and a low-fat spread.

When said foodstuff is a yoghurt, it may have any feature of the yoghurt described according to the ninth aspect.

5 When said foodstuff is an ice-cream type dessert, it may have any feature of the dessert described according to the ninth aspect.

10 When said foodstuff is a milk drink, it may have any feature of the drink described according to the ninth aspect.

15 When said foodstuff is a low fat spread, it may have any feature of the spread described according to the ninth aspect.

20 According to an eleventh aspect of the invention, there is provided the use of an edible fungus in the preparation of a foodstuff for human consumption, especially a dairy product (suitably so that said edible fungus, not solely an extract thereof is present in the foodstuff), for treatment of joint mobility disorders; for reducing fat uptake; for lowering cholesterol; for immune function stimulation; as a pre-biotic and/or for affecting
25 satiety.

30 According to a twelfth aspect of the invention, there is provided a method of preparing a foodstuff, especially a dairy product, having at least 300mg (preferably at least 350mg and suitably less than 600mg) of N-acetylglucosamine per 100g of foodstuff; at least 600mg (preferably at least 750mg and suitably less than 1300mg) of β -glucan per 100g of foodstuff.

The edible fungi and/or foodstuff of the eleventh and/or twelfth aspects may be as described in any statement herein. Preferably, said edible fungi is
5 adapted to act as a fat mimetic in said foodstuff.

Said foodstuff is preferably fluidic and/or spreadable.

10 Said foodstuff suitably includes less than 2% w/w, preferably less than 0.5% w/w, especially less than 0.25% w/w or even less than 0.1% w/w of egg albumin. Said, foodstuff preferably includes substantially no egg albumin.

15 Said edible fungi preferably comprise a filamentous fungus.

According to a thirteenth aspect, there is provided a
20 foodstuff, having at least 300mg of N-acetylglucosamine and at least 600mg of β -glucan per 100g of foodstuff.

Any feature of any aspect of any invention or embodiment described herein may be combined with any
25 feature of any aspect of any other invention or embodiment described herein mutatis mutandis.

Specific embodiments of the invention will now be described, by way of example, with reference to the
30 accompanying drawings, in which:

Figure 1 is a schematic representation of an APV Lab 2000 homogeniser;

Figure 2 is a principal component plot describing the attributes of an ice-cream type dessert;

5 The following are referred to hereinafter:

mycoprotein paste - refers to a visco-elastic material comprising a mass of edible filamentous fungus derived from *Fusarium venenatum* A3/5 (formerly classified as
10 *Fusarium graminearum* Schwabe) (IMI 145425; ATCC PTA-2684 deposited with the American type Culture Collection, 10801 University Boulevard Manassas, VA, US) and treated to reduce its RNA content to less than 2% by weight by heat treatment. Further details on the material are provided
15 in WO96/21362 and WO95/23843. The material may be obtained from Marlow Foods Limited of Stokesley, U.K. It comprises about 25 wt% solids made up of non-viable RNA reduced fungal hyphae of approximately 400-750 μm length, 3-5 μm in diameter and a branching frequency of 2-8 tips
20 per hyphal length.

Hobart mixer - a beater mixer with a planetary mixing action made by Hobart Corporation of Troy Ohio, U.S.A.

25 Silverson L4RT high shear blender - obtained from Silverson Machines Ltd of Bucks, England.

APV Lab 2000 homogeniser - supplied by APV Homogenisers AS of Denmark. It is a research and
30 development tool for exploring homogenisation at feed stream pressures of up to 2000 bar.

Crepaco homogeniser - supplied by APV Crepaco and capable of operating at a maximum feed stream pressure of 350 bar.

- 5 Kestner Lab Spray Dryer - Spray Dryer No 5 obtained from Kestner Evaporator & Engineer Co of London, England.

Stefan Mixer - supplied by Stephan Nahrungsmittel und Verfahrens Technik of Germany. For this mixer, the mixing
10 head is based on the speed of rotation of selected blade designs.

Modified starch (National Starch Coarse Instant Clear Jel) - a pre-gelatinised modified starch used as a
15 thickener obtained from National Starch.

Butter flavour 2807 - a mixture of flavouring substances obtained from Danisco Ingredients of Denmark.

Buttermilk powder - supplied by Dairy Crest of
20 Surbiton, U.K.

Pea protein isolate - obtained from ACP Ingredients Limited

Oil blend (Grinstead PS209) - blend of mono and triglycerides based on edible fully hydrogenated vegetable
25 oil obtained from Danisco Ingredients of Denmark.

Ytron mixer - a high shear mixer

Dimodan OT - an emulsifier comprising distilled monoglycerides of fatty acids supplied by Danisco Ingredients of Denmark.

30 Pectin Grinsted PS209 - a pectin thickener from Danisco.

Butter flavour 2822 - a mixture of flavouring substances obtained from Danisco Ingredients of Denmark.

Crepaco Scraped Surface Heat Exchanger - supplied by APV Crepaco. It provides a means by which product can be heated or cooled within a jacketed vessel and, at the same time, scraping the surface of the heat transfer contact point so as to prevent fouling.

Genupectin YM-100-L - a pectin thickener obtained from CP Kelco UK Ltd

Viscarin GP2050 - a carrageenan - based hydrocolloid obtained from FMC Biopolymer Ltd.

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Example 1 - the influence of dispersion time and mixing methodology on dispersion efficiency.

Mycoprotein paste was added to shop-bought skim milk at 25% w/w and left to 'hydrate' further for 5, 15 and 30 minutes. After each time interval the 'dispersion' was filtered using a coarse muslin cloth and the amount of residual solids quantified as a measure of degree of dispersion. In addition, at each time interval one batch of the dispersion was mixed for 4 minutes using a Hobart mixer on setting number 4 whilst a second batch was mixed using a Silverson L4RT high shear blender using a slotted disintegrating head at 8000rpm. In each case the dispersion efficiency was measured on the basis of residual solids in the muslin cloth.

Results are provided in Table 1.

Table 1

PROCESS	TIME (min)	% RESIDUAL SOLIDS
NO TREATMENT	5	95%
	15	94%
	30	94%
MIXING USING SILVERSON MIXER	5	20%
	15	15%
	30	8%
MIXING USING HOBART MIXER	5	60%
	15	42%
	30	30%

The experiments illustrate that it is beneficial to hydrate the mycoprotein paste prior to dispersion by agitation. Additionally, it is beneficial to use a high shear mixer (e.g. Silverson). Similar benefits were found for dispersions made in either 3% w/w caseinate solution or 3% w/w whey protein concentrate instead of skim milk. In general terms, any protein-containing aqueous liquid may be used.

Unless otherwise stated herein, when a formulation comprising mycoprotein paste and skim milk or water is used, the paste is allowed to hydrate for 30 minutes prior to subsequent use.

Example 2 - investigations relating to homogenisation.

The basic principles of homogenisation will be described with reference to figure 1. Unhomogenised product 2 enters the valve seat 4 at low viscosity and low

pressure. As the product flows through an adjustable close clearance area between a valve 6 and seat 4, there is a rapid increase in velocity with a corresponding decrease in pressure. This intense energy transition
5 occurs in microseconds and produces turbulent three dimensional mixing layers that disrupt particles at the discharge from the gap 8. The homogenised product (9) impinges on an impact ring 10 and exists at a pressure sufficient for movement to the next processing stage. The
10 acceleration of the liquids through the gap also produces a pressure drop to below the vapour pressure of some components. This may lead to implosive forces being generated.

15 Effect of homogenisation temperature on flowrate through homogeniser

The effect of homogenisation temperature on flow rate through the APV Lab 2000 homogeniser of the
20 mycoprotein/skim milk formulation described in Example 1 was assessed over a range of pressures and the results are provided in Table 2 wherein "1st stage" and "2nd stage" pressure refer to the pressure of the formulation when entering through valve seat 4 and the subsequent
25 downstream pressure (the pressure measured at the exit of the valve assembly) respectively.

Table 2

Temperature °C	2 nd stage pressure (bar)	1 st stage pressure (bar)	Q (kg/h)
20	0	0	15
20	90	500	8
20	230	1210	6
20	350	1600	5.4
50	90	500	11
50	230	1210	10
50	350	1600	10
70	90	500	8
70	230	1210	6.6
70	350	1600	6

It will be noted from Table 2 that the optimum flow rate is achieved at about 50°C.

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(b) Effect of dispersion and/or homogenisation processes on hyphal aspect ratio of the mycoprotein

10 Typically, mycoprotein hyphae are 400-750µm in length with a diameter of 3-5µm. The effect of a range of dispersion and/or homogenisation processes on the measured hyphal lengths of mycoprotein filaments was investigated. Details of processes used and the results are provided in
 15 Table 3a. In each case, a formulation was prepared of mycoprotein paste (25%w/w) and water or skim milk, with the paste being allowed to hydrate for 30 minutes prior to the subsequent processes described in the Table.

The assessment of hyphal lengths in a sample of mycoprotein is undertaken as follows: Light microscope preparations are made from the sample and light microscope images captured and processed as greyscale bitmaps. The
5 images are saved on 8-bit greyscale bitmaps to a resolution of 764-576 pixels. The magnification was determined as 0.81 micron/pixel using a static graticule and corresponding to a field of view of 0.62 x 0.47mm for each image. Dedicated software was written to analyse the
10 images.

15

20

Table 3a

Example No	Process	Mean (um)	Standard deviation (um)	Median (um)
2a	The formulation of mycoprotein paste in water was dispersed using a Silverson blender as described in Example 1	34.1	66.1	7.3
2b	The formulation of mycoprotein paste in water was dispersed using a Silverson blender as described in Example 1 followed by homogenisation using the Crepaco homogeniser at 270 bar			
2c	The formulation of mycoprotein paste in water was dispersed using a Silverson blender as described in Example 1 followed by homogenisation using the Crepaco homogeniser at 270 bar followed by homogenisation using the APV Lab 2000 homogeniser at an inlet pressure of 750 bar and a second stage pressure of 75 bar	18.3	20.5	12.1
2d	The formulation of mycoprotein paste in water was dispersed using a Silverson blender as described in Example 1 followed by homogenisation using the Crepaco homogeniser at 270 bar followed by homogenisation using the APV Lab 2000 homogeniser at an inlet pressure of 1500 bar and a second stage pressure of 350 bar.	15.7	15.7	11.3
2e	The formulation of mycoprotein paste in skim milk was dispersed using a Silverson blender as described in Example 1.	50.8	53.1	31.5
2f	The formulation of mycoprotein paste in skim milk was dispersed using a	26.5	52.2	13.7